

In the Claims:

1. (Withdrawn) A method of increasing a fraction of free carotenoids in a source of carotenoids in which at least some of the carotenoids are fatty acid esterified carotenoids, the method comprising contacting the source of carotenoids with an effective amount of an esterase under conditions effective in deesterifying the fatty acid esterified carotenoids, wherein said conditions effective in deesterifying the fatty acid esterified carotenoids are characterized by addition of at least one additive selected from the group consisting of:

a cellulose degrading enzyme;

a protein degrading enzyme;

a pectin degrading enzyme;

an emulsifier; and

at least one metal ion,

thereby increasing the fraction of free carotenoids in the source of carotenoids.

2. (Withdrawn) The method of claim 1, wherein said source of carotenoids is characterized in that a majority of the carotenoids in said source of carotenoids are said fatty acid esterified carotenoids.

3. (Withdrawn) The method of claim 1, wherein said source of carotenoids is red pepper.

4. (Withdrawn) The method of claim 1, wherein said source of carotenoids is red pepper powder.

5. (Withdrawn) The method of claim 1, wherein said source of carotenoids is paprika.

6. (Withdrawn) The method of claim 1, wherein said source of carotenoids is red pepper oil extract.

7. (Withdrawn) The method of claim 1, wherein said source of carotenoids is red pepper oleoresin.

8. (Withdrawn) The method of claim 1, wherein said source of carotenoids is selected from the group consisting of apple, apricot, avocado, blood orange cape gooseberry, carambola, chilli, clementine, kumquat, loquat, mango, minneola, nectarine, orange, papaya, peach, persimmon, plum, potato, pumpkin, tangerine and zucchini.

9. (Withdrawn) The method of claim 1, wherein said esterase is selected from the group consisting of a lipase, a carboxyl ester esterase and a chlorophylase.

10. (Withdrawn) The method of claim 1, wherein said esterase is a lipase.

11. (Withdrawn) The method of claim 10, wherein said lipase is selected from the group consisting of bacterial lipase, yeast lipase, mold lipase and animal lipase.

12. (Withdrawn) The method of claim 1, wherein said esterase is an immobilized esterase.

13. (Withdrawn) The method of claim 12, wherein said immobilized esterase is a recycled immobilized esterase.

14. (Withdrawn) The method of claim 1, wherein said at least one metal ion is selected from the group consisting of Ca^{++} and Na^+ .

15. (Withdrawn) The method of claim 1, wherein said addition of said at least one metal ion is by addition of at least one salt of said metal ion.

16. (Withdrawn) The method of claim 1, wherein said at least one salt is selected from the group consisting of CaCl_2 and NaCl .

17. (Withdrawn) The method of claim 1, wherein said cellulose degrading enzyme is selected from the group consisting of C1 type beta-1,4 glucanase, exo-beta-1,4 glucanase, endo-beta-1,4 glucanase and beta-glucosidase.

18. (Withdrawn) The method of claim 1, wherein said proteins degrading enzyme is selected from the group consisting of trypsin, papain, chymotrypsins, ficin, bromelin, cathepsins and rennin.

19. (Withdrawn) The method of claim 1, wherein said pectin degrading enzyme is selected from the group consisting of a pectinesterase, pectate lyase and a polygalacturonase.

20. (Withdrawn) The method of claim 1, wherein said emulsifier is a non-ester emulsifier.

21. (Withdrawn) The method of claim 1, wherein said emulsifier is lecithin.

22. (Withdrawn) The method of claim 20, wherein said emulsifier is deoxycholate.

23. (Withdrawn) The method of claim 1, wherein said emulsifier is a non-ionic detergent.

24. (Withdrawn) The method of claim 20, wherein said emulsifier is derived from bile, gum Arabic or salt of free fatty acids.

25. (Withdrawn) The method of claim 1, wherein said emulsifier is a recycled emulsifier.

26. (Withdrawn) The method of claim 1, further comprising extracting free carotenoids from the source of carotenoids.

27. (Withdrawn) The method of claim 26, wherein said extracting free carotenoids from the source of carotenoids comprises extracting with ethyl acetate under alkaline conditions

28. (Withdrawn) The method of claim 27, wherein said alkaline conditions are characterized by pH from about 8.0 to about 10.

29. (Withdrawn) The method of claim 28, wherein said alkaline conditions are pH 9.5.

30. (Withdrawn) A source of carotenoids having an increased fraction of free carotenoids and produced by the method of claim 1.

31. (Withdrawn) A food additive comprising the source of carotenoids of claim 30.

32. (Withdrawn) A feed additive comprising the source of carotenoids of claim 30.

33. (Withdrawn) A method of increasing a fraction of free carotenoids in a source of carotenoids in which at least some of the carotenoids are fatty acid esterified carotenoids, the method comprising contacting the source of carotenoids with an effective amount of an esterase under conditions effective in deesterifying the fatty acid esterified carotenoids, so as to produce a source of at least partially deesterified carotenoids, and extracting said source of at least partially deesterified carotenoids with ethyl acetate under alkaline conditions,

thereby increasing the fraction of free carotenoids in the source of carotenoids.

34. (Withdrawn) The method of claim 33, wherein said alkaline conditions are characterized by pH from about 8.0 to about 10.

35. (Withdrawn) The method of claim 33, wherein said alkaline conditions are pH 9.5.

36. (Withdrawn) The method of claim 33, wherein said source of carotenoids is characterized in that a majority of the carotenoids in said source of carotenoids are said fatty acid esterified carotenoids.

37. (Withdrawn) The method of claim 33, wherein said conditions effective in deesterifying the fatty acid esterified carotenoids are characterized by addition of at least one additive selected from the group consisting of: a cellulose degrading enzyme; a protein degrading enzyme; a pectin degrading enzyme; an emulsifier; and at least one metal ion.

38. (Withdrawn) The method of claim 33, wherein said source of carotenoids is red pepper.

39. (Withdrawn) The method of claim 33, wherein said source of carotenoids is red pepper powder.

40. (Withdrawn) The method of claim 33, wherein said source of carotenoids is paprika.

41. (Withdrawn) The method of claim 33, wherein said source of carotenoids is red pepper oil extract.

42. (Withdrawn) The method of claim 33, wherein said source of carotenoids is red pepper oleoresin.

43. (Withdrawn) The method of claim 33, wherein said source of carotenoids is selected from the group consisting of apple, apricot, avocado, blood orange cape gooseberry, carambola, chilli, clementine, kumquat, loquat, mango, minneola, nectarine, orange, papaya, peach, persimmon, plum, potato, pumpkin, tangerine and zucchini.

44. (Withdrawn) The method of claim 33, wherein said esterase is selected from the group consisting of a lipase, a carboxyl ester esterase and a chlorophylase.

45. (Withdrawn) The method of claim 33, wherein said esterase is a lipase.

46. (Withdrawn) The method of claim 45, wherein said lipase is selected from the group consisting of bacterial lipase, yeast lipase, mold lipase and animal lipase.

47. (Withdrawn) The method of claim 33, wherein said esterase is an immobilized lipase.

48. (Withdrawn) The method of claim 47, wherein said immobilized lipase is a recycled immobilized lipase.

49. (Withdrawn) The method of claim 37, wherein said at least one metal ion is selected from the group consisting of Ca^{++} and Na^+ .

50. (Withdrawn) The method of claim 37, wherein said addition of said at least one metal ion is by addition of at least one salt of said metal ion.

51. (Withdrawn) The method of claim 50, wherein said at least one salt is selected from the group consisting of CaCl_2 and NaCl .

52. (Withdrawn) The method of claim 37, wherein said cellulose degrading enzyme is selected from the group consisting of C1 type beta-1,4 glucanase, exo-beta-1,4 glucanase, endo-beta-1,4 glucanase and beta-glucosidase.

53. (Withdrawn) The method of claim 37, wherein said proteins degrading enzyme is selected from the group consisting of trypsin, papain, chymotrypsins, ficin, bromelin, cathepsins and rennin.

54. (Withdrawn) The method of claim 37, wherein said pectin degrading enzyme is selected from the group consisting of a pectin esterase, pectate lyase and a polygalacturonase.

55. (Withdrawn) The method of claim 37, wherein said emulsifier is a non-ester emulsifier.

56. (Withdrawn) The method of claim 37, wherein said emulsifier is lecithin.

57. (Withdrawn) The method of claim 55, wherein said emulsifier is deoxycholate.

58. (Withdrawn) The method of claim 37, wherein said emulsifier is a non-ionic detergent.

59. (Withdrawn) The method of claim 55, wherein said emulsifier is derived from bile, gum Arabic or salt of free fatty acids.

60. (Withdrawn) The method of claim 37, wherein said emulsifier is a recycled emulsifier.

61. (Withdrawn) A source of carotenoids having an increased fraction of free carotenoids and produced by the method of claim 33.

62. (Withdrawn) A food additive comprising the source of carotenoids of claim 61.

63. (Withdrawn) A feed additive comprising the source of carotenoids of claim 61.

64. (Withdrawn) A method of increasing a fraction of free carotenoids in a source of carotenoids in which at least some of the carotenoids are fatty acid esterified carotenoids, the method comprising contacting the source of carotenoids

with an effective amount of an immobilized esterase under conditions effective in deesterifying the fatty acid esterified carotenoids, thereby increasing the fraction of free carotenoids in the source of carotenoids.

65. (Withdrawn) The method of claim 64, wherein said immobilized esterase is selected from the group consisting of an immobilized lipase, an immobilized carboxyl ester esterase and an immobilized chlorophylase.

66. (Withdrawn) The method of claim 64, wherein said immobilized esterase is an immobilized lipase.

67. (Withdrawn) The method of claim 66, wherein said immobilized lipase is selected from the group consisting of an immobilized bacterial lipase, immobilized yeast lipase, immobilized mold lipase and immobilized animal lipase.

68. (Withdrawn) The method of claim 66, wherein said immobilized lipase is a recycled immobilized lipase.

69. (Withdrawn) The method of claim 64, wherein said source of carotenoids is characterized in that a majority of the carotenoids in said source of carotenoids are said fatty acid esterified carotenoids.

70. (Withdrawn) The method of claim 64, wherein said conditions effective in deesterifying the fatty acid esterified carotenoids are characterized by addition of at least one additive selected from the group consisting of: a cellulose degrading enzyme; a protein degrading enzyme; a pectin degrading enzyme; an emulsifier; and at least one metal ion.

71. (Withdrawn) The method of claim 64, wherein said source of carotenoids is red pepper.

72. (Withdrawn) The method of claim 64, wherein said source of carotenoids is red pepper powder.

73. (Withdrawn) The method of claim 64, wherein said source of carotenoids is paprika.

74. (Withdrawn) The method of claim 64, wherein said source of carotenoids is red pepper oil extract.

75. (Withdrawn) The method of claim 64, wherein said source of carotenoids is red pepper oleoresin.

76. (Withdrawn) The method of claim 64, wherein said source of carotenoids is selected from the group consisting of apple, apricot, avocado, blood orange cape gooseberry, carambola, chilli, clementine, kumquat, loquat, mango, minneola, nectarine, orange, papaya, peach, persimmon, plum, potato, pumpkin, tangerine and zucchini.

77. (Withdrawn) The method of claim 70, wherein said at least one metal ion is selected from the group consisting of Ca^{++} and Na^+ .

78. (Withdrawn) The method of claim 70 wherein said addition of said at least one metal ion is by addition of at least one salt of said metal ion.

79. (Withdrawn) The method of claim 78, wherein said at least one salt is selected from the group consisting of CaCl_2 and NaCl .

80. (Withdrawn) The method of claim 70, wherein said cellulose degrading enzyme is selected from the group consisting of C1 type beta-1,4 glucanase, exo-beta-1,4 glucanase, endo-beta-1,4 glucanase and beta-glucosidase.

81. (Withdrawn) The method of claim 70, wherein said proteins degrading enzyme is selected from the group consisting of trypsin, papain, chymotrypsins, ficin, bromelin, cathepsins and rennin.

82. (Withdrawn) The method of claim 70, wherein said pectin degrading enzyme is selected from the group consisting of a pectin esterase, pectate lyase and a polygalacturonase.

83. (Withdrawn) The method of claim 70, wherein said emulsifier is a non-ester emulsifier.

84. (Withdrawn) The method of claim 70, wherein said emulsifier is lecithin.

85. (Withdrawn) The method of claim 83, wherein said emulsifier is deoxycholate.

86. (Withdrawn) The method of claim 70, wherein said emulsifier is a non-ionic detergent.

87. (Withdrawn) The method of claim 83, wherein said emulsifier is derived from bile, gum Arabic or salt of free fatty acids.

88. (Withdrawn) The method of claim 70, wherein said emulsifier is a recycled emulsifier.

89. (Withdrawn) The method of claim 64, further comprising extracting said source of at least partially deesterified carotenoids with ethyl acetate under alkaline conditions.

90. (Withdrawn) The method of claim 89, wherein said alkaline conditions are characterized by pH from about 8.0 to about 10.

91. (Withdrawn) The method of claim 89, wherein said alkaline conditions are pH 9.5.

92. (Withdrawn) A source of carotenoids having an increased fraction of free carotenoids and produced by the method of claim 64.

93. (Withdrawn) A food additive comprising the source of carotenoids of claim 92.

94. (Withdrawn) A feed additive comprising the source of carotenoids of claim 92.

95. (Currently Amended) A method of increasing a fraction of free carotenoids in a source of carotenoids in which at least some of the carotenoids are fatty acid esterified carotenoids, the method comprising the steps of

(a) contacting the source of carotenoids with an effective amount of an esterase and a recycled emulsifier under conditions effective in deesterifying the fatty acid esterified carotenoids, wherein said conditions effective in deesterifying the fatty acid esterified carotenoids are characterized by addition of at least one additive selected from the group consisting of:

a cellulose degrading enzyme;

a protein degrading enzyme;

a pectin degrading enzyme; and

at least one metal ion; and

(b) extracting said source of at least partially deesterified carotenoids with ethyl acetate under alkaline conditions,

thereby increasing the fraction of free carotenoids in the source of carotenoids.

96. (Original) The method of claim 95, wherein said emulsifier is a non-ester emulsifier.

97. (Original) The method of claim 95, wherein said emulsifier is lecithin.

98. (Original) The method of claim 95, wherein said emulsifier is deoxycholate.

99. (Original) The method of claim 95, wherein said emulsifier is a non-ionic detergent.

100. (Original) The method of claim 96, wherein said emulsifier is derived from bile, gum Arabic or salt of free fatty acids.

101. (Original) The method of claim 95, wherein said source of carotenoids is characterized in that a majority of the carotenoids in said source of carotenoids are said fatty acid esterified carotenoids.

102. (Canceled)

103. (Original) The method of claim 95, wherein said source of carotenoids is red pepper.

104. (Original) The method of claim 95, wherein said source of carotenoids is red pepper powder.

105. (Original) The method of claim 95, wherein said source of carotenoids is paprika.

106. (Original) The method of claim 95, wherein said source of carotenoids is red pepper oil extract.

107. (Original) The method of claim 95, wherein said source of carotenoids is red pepper oleoresin.

108. (Original) The method of claim 95, wherein said source of carotenoids is selected from the group consisting of apple, apricot, avocado, blood orange cape gooseberry, carambola, chilli, clementine, kumquat, loquat, mango, minneola, nectarine, orange, papaya, peach, persimmon, plum, potato, pumpkin, tangerine and zucchini.

109. (Original) The method of claim 95, wherein said esterase is selected from the group consisting of a lipase, a carboxyl ester esterase and a chlorophylase.

110. (Original) The method of claim 95, wherein said esterase is a lipase.

111. (Original) The method of claim 110, wherein said lipase is selected from the group consisting of bacterial lipase, yeast lipase, mold lipase and animal lipase.

112. (Original) The method of claim 95, wherein said esterase is an immobilized lipase.

113. (Original) The method of claim 112, wherein said immobilized lipase is a recycled immobilized lipase.

114. (Currently Amended) The method of claim ~~102~~ 95, wherein said at least one metal ion is selected from the group consisting of Ca^{++} and Na^+ .

115. (Currently Amended) The method of claim ~~102~~ 95, wherein said addition of said at least one metal ion is by addition of at least one salt of said metal ion.

116. (Original) The method of claim 115, wherein said at least one salt is selected from the group consisting of CaCl_2 and NaCl .

117. (Currently Amended) The method of claim ~~102~~ 95, wherein said cellulose degrading enzyme is selected from the group consisting of C1 type beta-1,4 glucanase, exo-beta-1,4 glucanase, endo-beta-1,4 glucanase and beta-glucosidase.

118. (Currently Amended) The method of claim ~~102~~ 95, wherein said proteins degrading enzyme is selected from the group consisting of trypsin, papain, chymotrypsins, ficin, bromelin, cathepsins and rennin.

119. (Currently Amended) The method of claim ~~102~~ 95, wherein said pectin degrading enzyme is selected from the group consisting of a pectin esterase, pectate lyase and a polygalacturonase.

120. (Canceled)

121. (Currently Amended) The method of claim ~~120~~ 95, wherein said alkaline conditions are characterized by pH from about 8.0 to about 10.

122. (Currently Amended) The method of claim ~~120~~ 95, wherein said alkaline conditions are pH 9.5.

223. (Withdrawn) The method of claim 218, wherein said emulsifier is a recycled emulsifier.

224. (Withdrawn) The method of claim 201, wherein said carotenoids detection assay is a chromatography assay.

225. (Withdrawn) The method of claim 224, wherein said chromatography assay is selected from the group consisting of thin layer chromatography and high performance liquid chromatography.

226. (Withdrawn) A method of optimizing reaction conditions for increasing a fraction of free carotenoids in a source of carotenoids in which at least some of the carotenoids are fatty acid esterified carotenoids, via an esterase, the method comprising:

contacting the source of carotenoids with the esterase under different preselected experimental conditions; and

using a carotenoids detection assay for determining the efficiency of the esterase in increasing the fraction of the free carotenoids in the source of carotenoids under each of said different preselected experimental conditions, thereby optimizing the reaction conditions for increasing the fraction of free carotenoids in the source of

carotenoids in which at least some of the carotenoids are fatty acid esterified carotenoids via the esterase.

227. (Withdrawn) The method of claim 226, wherein said source of carotenoids is characterized in that a majority of the carotenoids in said source of carotenoids are said fatty acid esterified carotenoids.

228. (Withdrawn) The method of claim 226, wherein said source of carotenoids is red pepper.

229. (Withdrawn) The method of claim 226, wherein said source of carotenoids is red pepper powder.

230. (Withdrawn) The method of claim 226, wherein said source of carotenoids is paprika.

231. (Withdrawn) The method of claim 226, wherein said source of carotenoids is red pepper oil extract.

232. (Withdrawn) The method of claim 226, wherein said source of carotenoids is red pepper oleoresin.

233. (Withdrawn) The method of claim 226, wherein said source of carotenoids is selected from the group consisting of apple, apricot, avocado, blood orange cape gooseberry, carambola, chilli, clementine, kumquat, loquat, mango, minneola, nectarine, orange, papaya, peach, persimmon, plum, potato, pumpkin, tangerine and zucchini.

234. (Withdrawn) The method of claim 226, wherein said esterase is selected from the group consisting of a lipase, a carboxyl ester esterase and a chlorophylase.

235. (Withdrawn) The method of claim 234, wherein said esterase is a lipase.

236. (Withdrawn) The method of claim 235, wherein said lipase is selected from the group consisting of bacterial lipase, yeast lipase, mold lipase and animal lipase.

237. (Withdrawn) The method of claim 226, wherein said esterase is an immobilized esterase.

238. (Withdrawn) The method of claim 237, wherein said immobilized esterase is a recycled immobilized esterase.

239. (Withdrawn) The method of claim 234, wherein said different preselected experimental conditions comprise at least one of:

- addition of a cellulose degrading enzyme;
- addition of a proteins degrading enzyme;
- addition of a pectin degrading enzyme; and
- addition of an emulsifier.

240. (Withdrawn) The method of claim 239, wherein said cellulose degrading enzyme is selected from the group consisting of C1 type beta-1,4 glucanase, exo-beta-1,4 glucanase, endo-beta-1,4 glucanase and beta-glucosidase.

241. (Withdrawn) The method of claim 239, wherein said proteins degrading enzyme is selected from the group consisting of trypsin, papain, chymotrypsins, ficin, bromelin, cathepsins and rennin.

242. (Withdrawn) The method of claim 239, wherein said pectin degrading enzyme is selected from the group consisting of a pectin esterase, pectate lyase and a polygalacturonase.

243. (Withdrawn) The method of claim 239, wherein said emulsifier is a non-ester emulsifier.

244. (Withdrawn) The method of claim 239, wherein said emulsifier is lecithin.

245. (Withdrawn) The method of claim 243, wherein said emulsifier is deoxycholate.

246. (Withdrawn) The method of claim 239, wherein said emulsifier is a non-ionic detergent.

247. (Withdrawn) The method of claim 243, wherein said emulsifier is derived from bile, gum Arabic or salt of free fatty acids.

248. (Withdrawn) The method of claim 239, wherein said emulsifier is a recycled emulsifier.

249. (Withdrawn) The method of claim 226, wherein said carotenoids detection assay is a chromatography assay.

250. (Withdrawn) The method of claim 249, wherein said chromatography assay is selected from the group consisting of thin layer chromatography and high performance liquid chromatography.

251. (Withdrawn) A method of extracting red pepper oleoresin, the method comprising:

- homogenizing red-pepper fruits in water into a juice;
- centrifuging the juice so as to obtain a pellet;
- mixing the pellet with ethanol and ethyl acetate;
- homogenizing the pellet with the ethanol and the ethyl acetate;
- removing dry material; and
- evaporating solvents so as to obtain red pepper oleoresin.

252. (Withdrawn) The method of claim 251, wherein a weight ratio between said red-pepper fruits and said water is 80-120 parts of fruit to 20 – 60 parts of water.

253. (Withdrawn) The method of claim 251, wherein said red-pepper fruits are frozen.

254. (Withdrawn) The method of claim 251, wherein said red-pepper fruits are fresh.

255. (Withdrawn) The method of claim 251, wherein said juice is centrifuged at 20,000 – 30,000 g for 10 – 30 minutes.

256. (Withdrawn) The method of claim 251, wherein said pellet is mixed with 1-3 parts of said ethanol and 5-15 parts of said ethyl acetate.

257. (Withdrawn) The method of claim 251, wherein said removing dry material is by centrifugation.

258. (Withdrawn) The method of claim 251, wherein said evaporating solvents is at 40-50 °C.

259. (Withdrawn) The method of claim 251, wherein said evaporating solvents is at 40-50 °C and under vacuum.

260. (Withdrawn) The method of claim 251, wherein said evaporating solvents is under vacuum.